

3rd lecture: ENZYMES



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ENZYMES

A many proteins are known with different biological functions:

- Regulator proteins
- Transport proteins
- Protecting proteins
- Toxins
- Reserve proteins
- Contractile proteins
- Structural proteins

ENZYMES - catalysts of reactions

$\epsilon \nu \zeta \upsilon \mu \eta$ = "in yeast" (greek) 1878 Kühne



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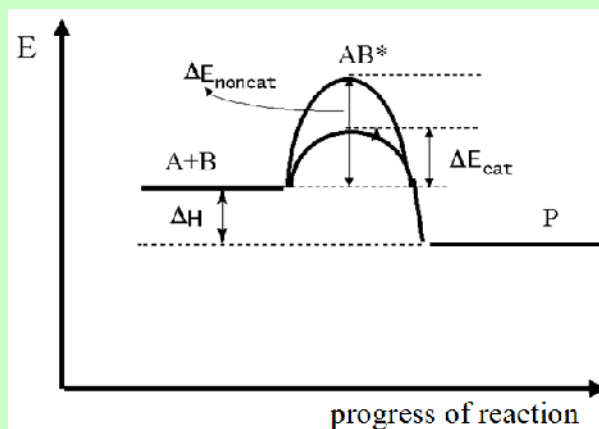
THERMODYNAMICS OF CATALYSIS

1930- years: Eyring:
During the reaction a higher energy transition complex is formed - activation energy (E^*) is needed:

$$k_r = \frac{kT}{h} e^{\frac{\Delta S^*}{R}} \cdot e^{-\frac{\Delta H^*}{RT}} \approx \text{const} \cdot e^{-\frac{\Delta E^*}{RT}}$$

k_r – reaction rate constant
T - absolute temperature (Kelvin)
k - Boltzmann constant (1,37.10-23 J/°K)
h - Planck constant (6,62.10-34 Js)

This energy is reduced by catalysts – the reaction rate is higher but the chemical equilibrium is not affected.



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Comparison of chemical and enzymatic catalysis

Reaction	Catalyst	Activation energy kJ/mol	k_{rel} 25 °C
$H_2O_2 \rightarrow H_2O + 1/2O_2$	-	75	1
	I^{-1}	56,5	$2,1 \cdot 10^3$
	catalase	26,8	$3,5 \cdot 10^8$
Casein + nH_2O (n+1) peptide	H^+	86	1
	trypsin	50	$2,1 \cdot 10^6$
Sucrose + H_2O glucose+fructose	H^+	107	1
	invertase	46	$5,6 \cdot 10^{10}$
Linoleic acid + O_2 linolene peroxide	-	150-270	1
	Cu^{2+}	30-50	$\sim 10^2$
	lipoxygenase	16,7	$\sim 10^7$



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Catalysis

General cases of the enzymatic catalysis
(taken from general chemistry):

1. acid-base catalysis
2. covalent catalysis
3. metal ion catalysis

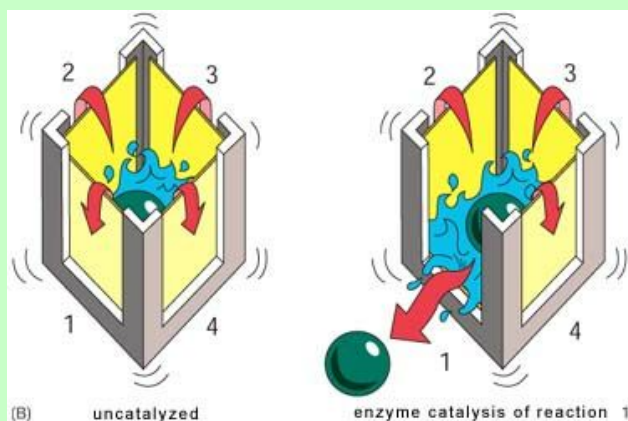


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ENZYMES

In a cell the organic compounds may react on many different way – but these reactions are very slow because of the activation energy barrier. The enzymes open a certain reaction route.



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Enzyme-substrate complex

A higher energy transition complex is formed:



The substrate attached to the **substrate binding site**, that is only a small portion of the surface of the enzyme molecule (sack/pocket).

Other domains on the surface:

- Catalytic domain = **ACTIVE CENTER** – the site for chemical reaction
- Sites for modulators (inhibitors, activators, S, P, metal ions)
- Sites for covalent modification of enzyme (phosphorylation, glycosylation, proteolysis)

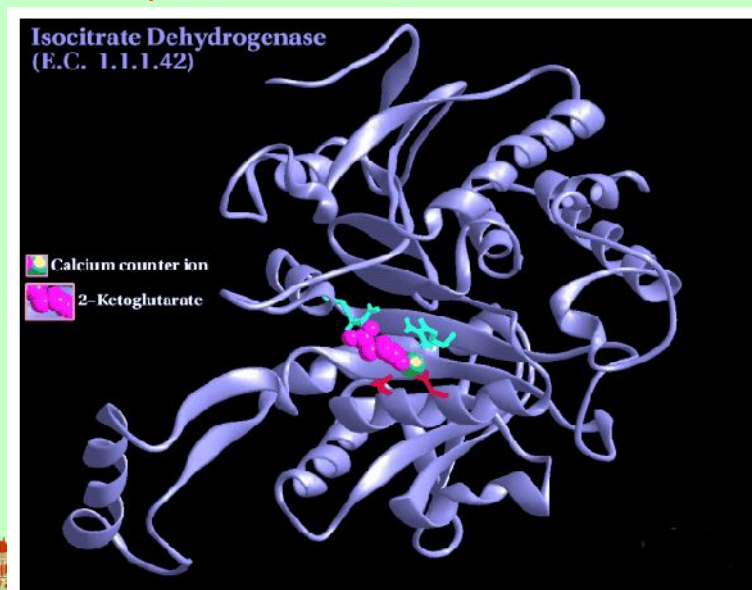


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Substrate binding site

The substrate binding site is only a small spot/pocket on the surface of enzyme molecule



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Enzyme-substrate interactions

... between the molecular surfaces:

Secondary (noncovalent) interactions:

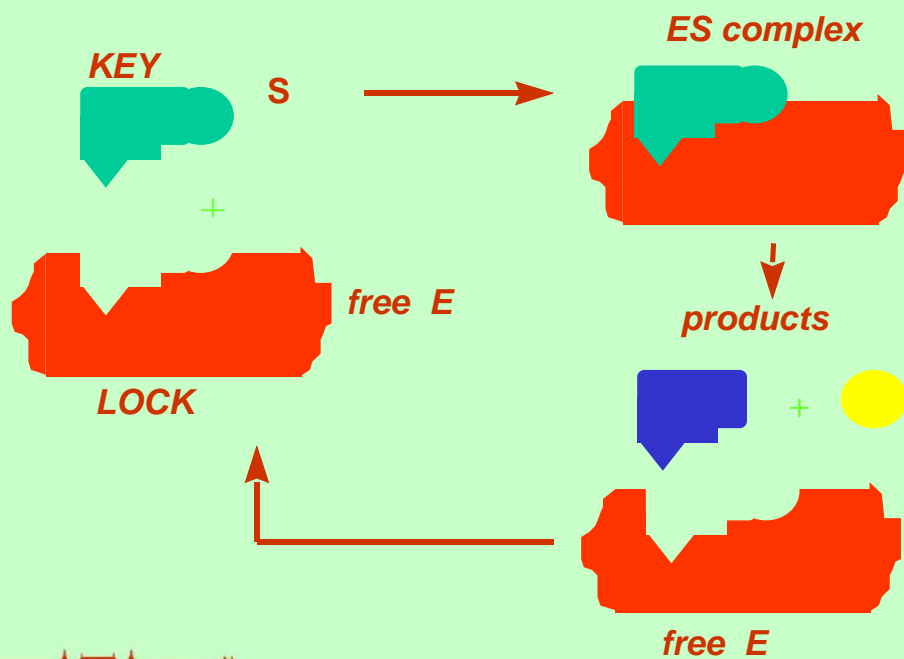
- electrostatic
- Van der Waals and
- hydrophobic interactions

Effects in enzyme-catalysis:

- lock and key model
- proximity effect
- orientation effect
- induced fit (Koshland-conformation change)



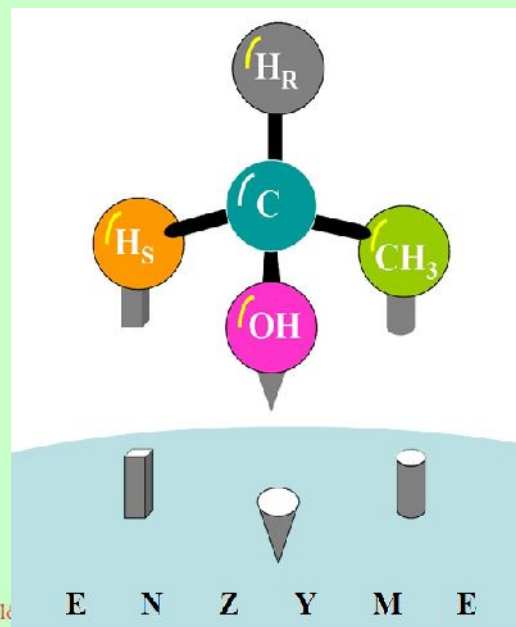
Lock and key model



Orientation effect

„Three-point attachment”: at least three functional groups of the substrate molecule bind to the enzyme - precise positioning, no rotation.

Only the proper optical isomer can attach – this is the base of **stereospecificity**.

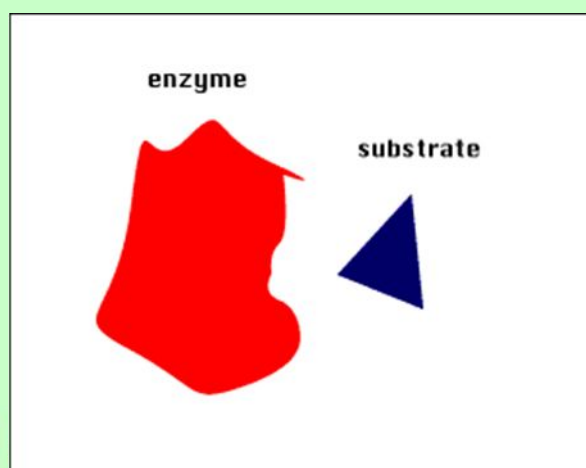


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Induced fit

In close approach (proximity) the form of the protein changes in interaction (Koshland, 1958), tends to complementarity and catches the substrate.

http://www.chem.ucsb.edu/~molvisual/ABLE/induced_fit/index.html

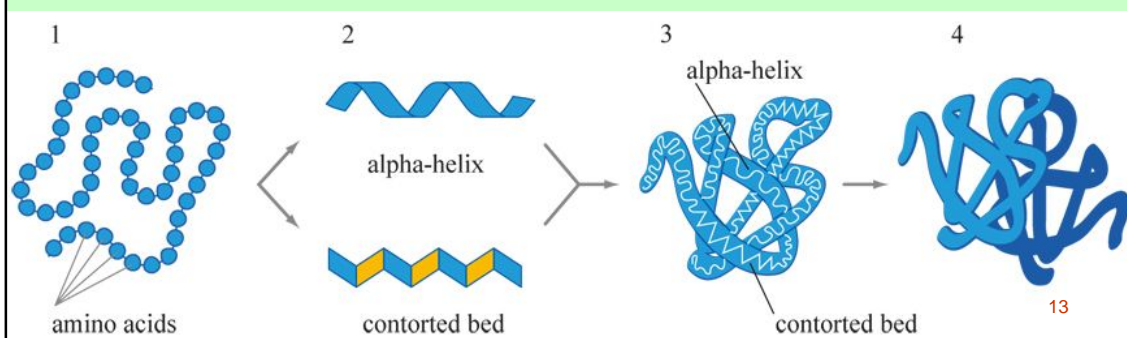


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How is the proper surface formed?

The folded peptide chains form the three dimensional structure of protein (tertiary, quaternary structure). The side chains of amino acids can be:

- apolar (alkyl groups)
- polar (-OH, -SH groups)
- ionic (-NH₂, -COOH groups)



Reactive side chains

Acidic: -COOH: Asp, Glu Basic: -NH₂: Lys, Arg
 terminal -COOH and -NH₂

Amide: -CO-NH₂: Asn, Gln

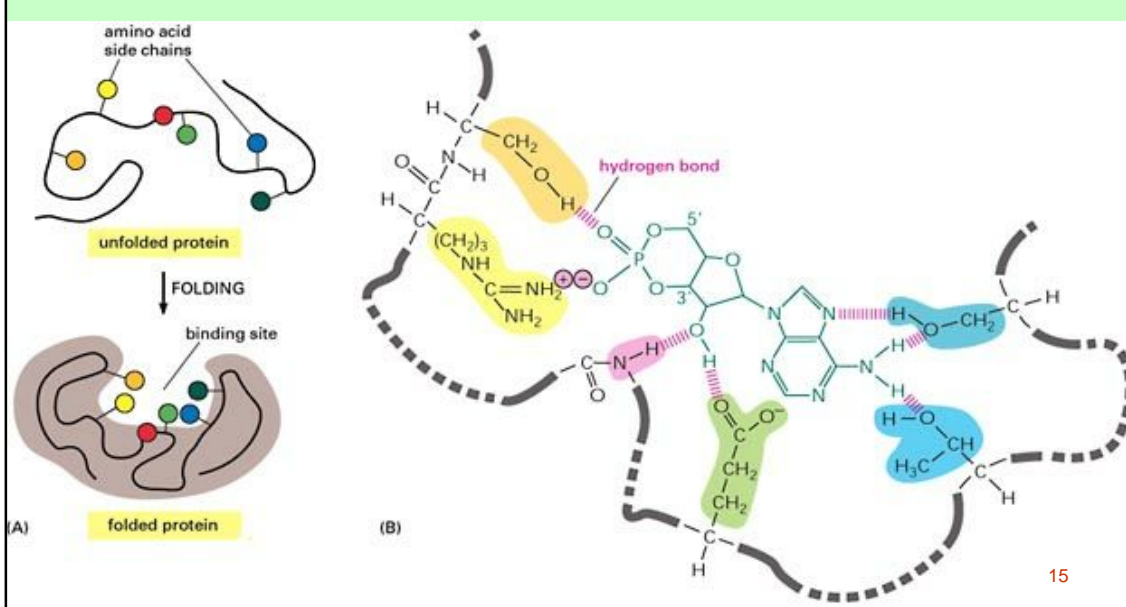
Polar: -OH: Ser, Thr -SH: Cys, -S-CH₃: Met

Imidazole: His Guanidine: Arg

H-bonds: C=O H-O- C=O H-NH-



Conformation of active center



Enzyme catalysed reactions

Only thermodynamically possible reactions can be catalysed
 $\Delta G < 0$

All enzyme catalysed reactions are reversible, tends to an equilibrium. but: the equilibrium can be shifted, e.g.. with product removal.

Proteins are denaturable: t, pH, ionic strength (salting out), organic solvents

Specificity: substrate-specificity
 group-specificity
 stereo-specificity
 region-specificity



Pros for enzyme catalysed reactions

Higher reaction rate: even 10^6 - 10^{12} x faster

Mild reaction condition (temperature, pressure, pH)

Sophisticated selectivity, better than in organic chemistry

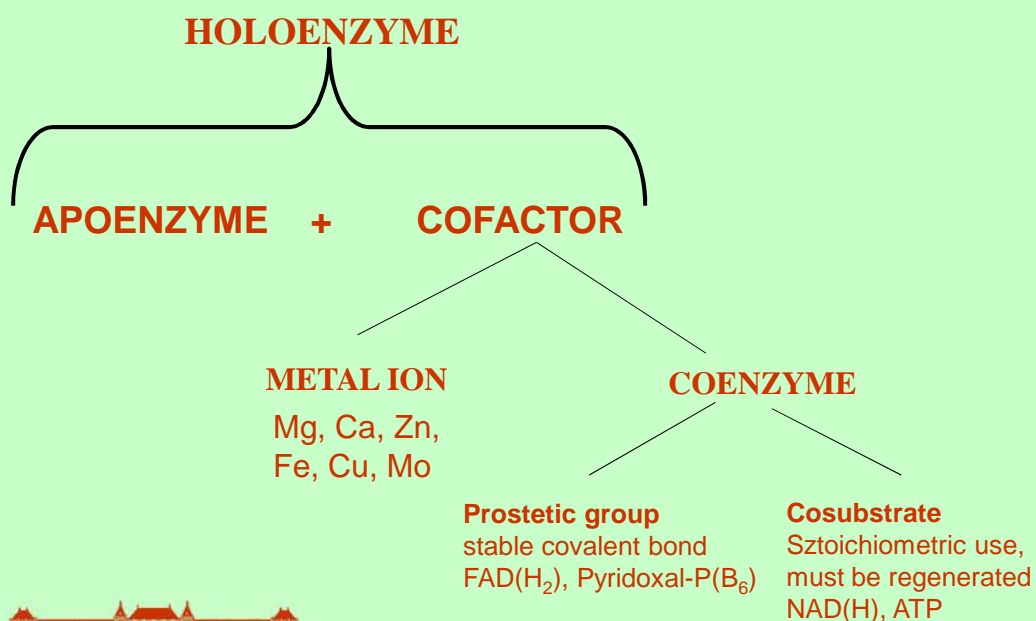
Easy control



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Necessary reaction partners



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Nomenclature of enzymes

1. To substrate:



urease

S-name + ase

2. To substrate and reaction: EtOH $\xrightarrow{\quad}$ AcO $\xrightarrow{\quad}$ AcOH

alcohol-dehydrogenase

S-name + reaction name + ase

3. Trivial names:

pepsin, trypsin, rennin – all peptidases

+ -in

4. IUB, IUPAC, IUBMB 1964, 1972, 1978 Enzyme Commission:
systematical nomenclature



Nomenclature of enzymes

catalogue number

cosubstrate

E.C.1.1.1.49. D-glucose-6P: NADP 1-oxydoreductase

the reaction

substrate

target on the 1st C-atom



Group	Reaction catalyzed	Typical reaction	Enzyme example(s) with trivial name
EC 1 Oxidoreductases	To catalyze oxidation/reduction reactions; transfer of H and O atoms or electrons from one substance to another	$AH + B \rightarrow A + BH$ (reduced) $A + O \rightarrow AO$ (oxidized)	Dehydrogenase, oxidase
EC 2 Transferases	Transfer of a functional group from one substance to another. The group may be methyl-, acyl-, amino- or phosphate group	$AB + C \rightarrow A + BC$	Transaminase, kinase
EC 3 Hydrolases	Formation of two products from a substrate by hydrolysis	$AB + H_2O \rightarrow AOH + BH$	Lipase, amylase, peptidase
EC 4 Lyases	Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved	$RCO_2COOH \rightarrow RCOH + CO_2$ or $[X-A-B-Y] \rightarrow [A=B + X-Y]$	Decarboxylase
EC 5 Isomerases	Intramolecule rearrangement, i.e. isomerization changes within a single molecule	$AB \rightarrow BA$	Isomerase, mutase
EC 6 Ligases	Join together two molecules by synthesis of new C-O, C-S, C-N or C-C bonds with simultaneous breakdown of ATP	$X + Y + ATP \rightarrow XY + ADP + P_i$	Synthetase

